Saleh D, Fournier E, Tharreau D. 2011. Experimental evolution induces loss of female-fertility in vitro in the fungal pathogen of rice, *Magnaporthe oryzae*.

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Sexual reproduction is widespread in eukaryotic organisms. Inmany fungal species previously known to reproduce asexually, some populations have been shown to encounter episodes of sexual reproduction. It has been suggested that mostfungi that are pathogenic on plants may reproduce sexually near their center of origin but have lost this ability during their dispersion. If a population reproduces exclusively clonally forseveral generations, individuals may accumulate deleterious mutations in loci controlling sexual reproduction. This may result in a complete loss of sexual reproduction ability. We tested this hypothesis on */Magnaporthe oryzae/*, which is responsible for the most important fungal disease on cultivated rice worldwide: blast. In this species, only asexual spores are observed in the field. However, some strains coming from the putative center of origin of the fungus (in South East Asia, near the Himalayan Foothills) are able to produce viable sexual spores */in vitro/*, and indirect evidence that sexual reproduction do occur /in natura/ in this region have been recently provided (see the abstract by E. Fournier).//In this heterothallic fungus,//sexual reproduction can occur only between two different strains of opposite mating type. Moreover, at least one of them, independently from the mating type, must be female-fertile, that is to be able to produce the sexual organs (perithecia) where meiosis takes place.

We performed /in vitro /experimental evolution of four female-fertile strains for 10-20 “clonal generations”. These strains came from a recombinant population of the center of origin. Each of the four strains became female-sterile in about 100 days in at least one of the two replicates we performed. This loss of female-fertility was accompanied by a reduction of asexual sporulation */in vitro /and /in planta/*.//As epigenetical effects could explain the frequent loss of female-fertility observed, we tested if stress could restore it. Four different stresses were tested: extremely low temperature (-80°C), mycelium fragmentation by sonication, monosporic isolation, growth on plant. None of these stresses achieved to restore female-fertility, so the loss of female-fertility might be explained by genetical rather than epigenetical mechanisms. Crosses were performed between mutant strains that had lost female-fertility and wild type strains. In the offspring, the female-fertility phenotype segregated in 1:3 proportions. Backcrosses between F1 female-sterile strains and wild type strains gave the same result, supporting the hypothesis of a genetic control.

Here we showed for the first time that female-fertility could be rapidly lost in the absence of sexual reproduction in */M. oryzae/* strains from rice. We showed that it might be due to mutation accumulation, probably at several loci. From these results we hypothesize that sexual reproduction ability may have been lost rapidly during the dispersion of the disease from Asia to the rest of the world. More generally, this study provides a case study to test hypotheses on the enigma of sex.